REMARKS

Reconsideration of the rejections set forth in the Office action mailed January 12, 2000 is respectfully requested. Claims 1 and 20-31 are currently under examination.

I. Amendments

The specification has been amended to correct minor typographical errors and to replace the existing Sequence listing with the corrected Sequence listing filed in the parent application.

No new matter is added by any of the amendments.

II. Rejections under 35 U.S.C. §102(e)

Claims 20, 21, and 26 were rejected under 35 U.S.C. §102(e) as being anticipated by Nikiforov *et al.* (U.S. Patent No. 5,679,524; "Nikiforov"). These rejections are respectfully traversed for the following reasons.

The standard for lack of novelty, that is, for anticipation, is one of strict identity. *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F2d 1367, 231 USPQ 81, 90 (Fed. Cir. 1986); *In re Donohue*, 766 F2d 531, 226 USPQ 619, 621 (Fed. Cir. 1985). To anticipate a claim for a patent, a single prior source must contain all its essential elements.

A. The Invention

The applicant's invention, as embodied in claim 1, is directed to a method for identifying a sequence of nucleotides in a polynucleotide. The method includes the steps of:

(a) extending an initializing oligonucleotide along the polynucleotide, by ligating an oligonucleotide probe thereto to form an extended duplex;

(b) identifying one or more nucleotides of the polynucleotide; and

(c) repeating steps (a) and (b) until the sequence of nucleotides is determined.

In accordance with the claim, the step of ligating an oligonucleotide probe to an initializing oligonucleotide alternates with the step of identifying one or more nucleotides in the polynucleotide template. These alternating steps are repeated until the sequence of nucleotides in the polynucleotide is determined.

B. The Prior Art

Nikiforov teaches a method of determining the identity of a single preselected site in a nucleic acid molecule (see e.g. column 1, lines 15-19). Specifically, this site is the site of a single nucleotide polymorphism (SNP); that is, the substitution of a single nucleotide in a gene sequence in some members of a species (see e.g. column 2, lines 34-43). Such substitutions are often characteristic of genetic disorders.

The SNP is identified, according to the method of Nikiforov, by hybridizing first and second oligonucleotides to the nucleic acid molecule, such that they are separated by one nucleotide, opposite the

SNP site (column 6, lines 13-20). The first and second oligonucleotides are then ligated, such that a deoxynucleoside triphosphate is incorporated at this site. Only a deoxynucleoside triphosphate complementary to the SNP nucleotide will be incorporated (column 6, lines 30-34), thus permitting identification of the SNP nucleotide.

The reference does not teach "repeating steps (a) and (b) until the sequence of nucleotides is determined", as recited in the present claim. Because the prior art method identifies only a single nucleotide, not a sequence of nucleotides, there would be no need for such repetition.

Since the reference does not disclose all of the elements set out above in claim 1, the claim cannot be anticipated by this reference under 35 U.S.C. §102(e). In view of this, the applicant respectfully requests the Examiner to withdraw the rejections under 35 U.S.C. §102(e).

III. Rejections under 35 U.S.C. §102(b) / 103

Claims 20, 21, and 26 were rejected under 35 U.S.C. §102(b) as being anticipated, or, alternatively, under 35 U.S.C. §103 as being obvious over, Pease *et al.* (*PNAS USA* **91**:5022, 1994). These rejections are respectfully traversed for the following reasons.

A. The Invention

The applicant's invention, as embodied in claim 20, provides a kit for DNA sequence analysis, useful in the methods for identifying polynucleotide sequences described in the application. The kit includes one or more sets of oligonucleotide probes, where (i) each probe set contains at least 50 different-sequence, single-stranded oligonucleotides, (ii) the oligonucleotides have lengths up to 12 nucleotides, and (iii) in each set, the different-sequence, single-stranded oligonucleotides within that set have substantially the same free energy of duplex formation.

In the kits of claims 21 and 26, each probe set contains from 50 to 10,000 different different-sequence, single-stranded oligonucleotides. In the kit of claim 26, the oligonucleotides are 8 to 12 nucleotides in length, and, in each set, the different-sequence, single-stranded oligonucleotides within that set are from the same stringency class.

The benefits of using sets of probes having substantially the same free energy of duplex formation, or from the same stringency class, are described at page 16, lines 11-22 of the specification. As noted at page 9, first paragraph, the probes are preferably applied to the polynucleotide template as mixtures comprising oligonucleotides of all possible sequences of a given length. A single such mixture can be quite complex; for example, the full set of 8-mers includes over 65,000 oligonucleotides. In a single such mixture, individual probes may not be present at concentrations sufficient to drive hybridization at a reasonable rate, particularly for sequences having lower free energy of binding.

The complexity can be reduced by the use of degeneracy-reducing analogs and/or by diving the mixture

into groups. As described at page 16, lines 11-21, by employing groups of stringency classes, each set of oligonucleotide probes from a single stringency class may be separately combined with the target polynucleotide under conditions such that substantially only oligonucleotide probes complementary to the target polynucleotide form duplexes. That is, the stringency of the hybridization reaction can be tailored to the free energy of duplex formation of the stringency class being used, so that substantially only perfectly complementary oligonucleotide probes form duplexes.

B. The Prior Art

Pease *et al.* describe oligonucleotide arrays prepared on a substrate for use in hybridization-based sequence analysis (SBH, or sequencing by hybridization). In a first illustrative example, a "complete set of octanucleotide probes", that is, 65,536 probes, is used (page 5022, 1st column, last paragraph). The next column refers to a paper by Southern (reference 7) employing 256 octanucleotides. As the appended abstract shows, this set constituted a complete set of octapurine (A/T) sequences. The "matrix of 256 spatially defined oligonucleotide probes" described in the next paragraph of Pease *et al.* is a complete set of tetranucleotides, as stated in the caption to Fig. 4. Finally, the Discussion section (first paragraph, pages 5025-6) refers to the "complete set" of octanucleotides, the "entire set" of dodecamers, the "set of 1,048,576 dodecanucleotides", and ways of placing such a complete set on a single substrate. There is no teaching or suggestion to provide subsets of oligonucleotides having similar free energy of duplex formation.

The Examiner refers specifically to the example described at page 5024, column 1, fifth paragraph, commenting that "most of the oligonucleotides in an array can be efficiently used". However, as described in this paragraph and the preceding paragraph, this substrate contained a single oligonucleotide probe (3'-CGCATCCG), not an array, generated at selected regions of the substrate, which were activated for synthesis by illumination through a mask. Compare the following example, in which two different sequences (3'-CGCATCCG and 3'-CGCTTCCG) were "synthesized in 800 x 12800 μm stripes" (column 2, second full paragraph).

Thus, the reference describes preparation of probe matrices which contain either a single sequence, two different sequences, or a complete set of all possible sequences of a given length. There is no teaching or suggestion to employ subsets of oligonucleotides defined, by their free energy of duplex formation, as stringency classes, as in the present claims.

Since the reference does not disclose or suggest all of the elements set out above in claims 20 and 26 and present in claim 21, the claims are cannot be anticipated, or rendered obvious, by this reference.

In view of this, the applicant respectfully requests the Examiner to withdraw the rejections under 35 U.S.C. §102(b)/103.

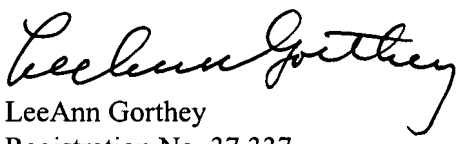
III. Conclusion

In view of the foregoing, the applicant submits that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 324-0880.

No further fees are believed necessary with this communication. However, the Commissioner is hereby authorized and requested to charge any deficiency in fees herein, or credit any overpayment, to Deposit Account No. 04-0531.

Respectfully submitted,



Date: 5-12-00

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